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(54) ATTENUATED CHICKEN-POX VIRUS OKA STRAIN GENE 62 AND METHOD FOR IDENTIFYING VIRUS STRAIN FOR ATTENUATED LIVE CHICKEN-POX VACCINE BY USING THE GENE

(57) The present application provides the gene 62 of Oka vaccine virus, which comprises at least with the following base substitutions (a) to (d) in the nucleotide sequence of SEQ ID No. 1: (a) the base A at position 2110 is G; (b) the base A at position 3100 is G; (c) the base T at position 3818 is C; and (d) the base A at position 4006 is G, and a method for identifying the Oka vaccine virus and an attenuated varicella-zoster virus strain acceptable as virus strain for live attenuated varicella vaccine, by using the gene 62.

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Description

Technical Field

[0001] The present application relates to gene 62 of the Oka vaccine virus and a method for identifying a virus strain for live attenuated varicella-zoster vaccine (referred to as "attenuated varicella-zoster virus strain" hereinafter) using the DNA sequence of gene 62. More specifically, the application relates to the gene 62 for use in discriminating the Oka vaccine virus and an attenuated varicella-zoster virus strain acceptable as the effective component of live attenuated vaccine for varicella-zoster, from the wild-type Oka vaccine virus (referred to as "virulent parental strain" or "parental strain" hereinafter) and other wild-type varicella-zoster virus strains; and a method for identifying an attenuated varicella-zoster virus, based on the nucleotide sequence of the gene 62.

Background Art

[0002] Varicella-zoster virus (abbreviated as VZV; referred to as "varicella virus" or "VZV" hereinafter) is the pathogenic virus of varicella and zoster in humans. Virus derived from "Oka vaccine virus" (Japanese Patent Publication No. 41202/1978; Lancet 2: 1288-1290, 1974) as one attenuated varicella-zoster virus strain has been used as the single unique seed for preparing live attenuated varicella vaccines, which are widely used worldwide [Requirements for Varicella Vaccine (Live) adopted 1984: WHO Technical Report Series, No. 725, pp 102-124, 1985].

[0003] Currently, the safety and effectiveness of the live attenuated varicella vaccines is certificated by the regulation of the passage number of the varicella seed virus with approval of the preparation (seed lot system). More specifically, so as to avoid the risk of genetic modification of the attenuated virus over continuous passages of the seed virus so that the resulting virus resumes the pathogenicity, it is defined under regulation that under provision that the passage number of the seed when approved is designated 0, virus within the total passage number of 10 from the passage number 0 is essentially used for such vaccines. In other words, the quality control and quality certificate of the live attenuated varicella vaccines depend on the observance of the seed lot system by each vaccine manufacture.

[0004] Meanwhile, no clinical reaction emerges in healthy children after the inoculation of the live attenuated varicella vaccines. The onset of varicella or zoster only rarely occurs in inoculated subjects with disorders of the immune function. However, whether or not such clinical reaction is due to the vaccination or spontaneous infection with wild-type varicella virus has never been identified. Thus, it is important epidemiologically to analyze the differentiation of the Oka vaccine virus as the seed virus of the live attenuated varicella vaccine from other wild-type VZV strains. Hence, a number of methods for the analysis have been proposed. Since the structure of the VZV genomic genes was reported (Journal of General Virology, 67: 1759-1816, 1986), methods have been proposed, for example, on the basis of the difference in DNA nucleotide sequence among VZV strains (Journal of Virology, 59: 660-668, 1986), the presence or absence of restriction enzyme *PstI* site (Japanese Journal of Experimental Medicine, 59: 233-237, 1989), determination based on RFLP (restriction fragment length polymorphism) by PCR (polymerase chain reaction) (Journal of Virology, 66: 1016-1020, 1992), and a combination of the presence or absence of the *PstI* site and the RFLP analysis of PCR products (Journal of Clinical Microbiology, 33:658-660, 1995). However, these methods are at low reliability when used singly. Thus, it has been difficult to definitely differentiate the Oka vaccine virus from other wild-type VZV strains.

[0005] Alternatively, the applicants of the present application have made diverse gene analysis of the Oka vaccine virus and other varicella virus strains (for example, known wild-type strains and wild-type strains newly isolated from patients with spontaneously infected varicella, and the like), and have identified eight requirements to differentiate the two kinds of strain types. Further the applicants have found that an unknown virus is acceptable as live varicella vaccine only when the virus satisfies all the eight requirements, and they have made a patent application based on the finding (WO 97/43420; referred to as "previous invention" hereinafter).

[0006] The method of the previous invention can discriminate the Oka vaccine virus from other varicella-zoster virus strains, but can never discriminate the Oka vaccine virus from the parental strain. Because all the eight required indicators are essentially examined, disadvantageously, the method needs a lot of time and labors until the discrimination is established.

[0007] The inventors of the present application have made investigations about a method for possibly differentiating the Oka vaccine virus from the parental strain, and they have found that the Oka vaccine virus, the parental strain and other wild-type varicella virus strains can be differentiated from each other in a simple and reliable manner, by detecting the mutation of varicella virus gene 62.

[0008] The invention of the present application has been attained on the basis of such novel finding, and a purpose of the invention is to provide the Oka vaccine virus-specific gene 62.

[0009] It is an additional purpose of the invention to provide the protein encoded by the gene 62 and a peptide thereof, an antibody and cytotoxic T lymphocyte (abbreviated as CTL) prepared by using them as antigen, and an attenuated varicella-zoster virus strain carrying the gene 62.

[0010] It is a still additional purpose of the invention to provide a method for identifying an attenuated varicella-zoster virus strain using the presence or absence of the mutation of the gene 62 as an indicator, and a live attenuated varicella vaccine prepared by using the attenuated virus strain identified by the method as the seed virus.

Disclosure of Invention

[0011] The present application provides the following individual inventions to satisfy the aforementioned purposes.

(1) Gene 62 of Oka vaccine virus, which comprises at least the following base substitutions (a) to (d) in the nucleotide sequence of SEQ ID No. 1:

(a) the base A at position 2110 is G;
(b) the base A at position 3100 is G;
(c) the base T at position 3818 is C; and
(d) the base A at position 4006 is G.

(2) The gene 62 of the Oka vaccine virus of the above (1), which comprises at least one or more of the following base substitutions (e) to (g) in addition to the base substitutions (a) to (d):

(e) the base A at position 1251 is G;
(f) the base A at position 2226 is G; and
(g) the base A at position 3657 is G.

(3) The gene 62 of the Oka vaccine virus of the above (2), which comprises at least one or more of the following base substitutions (h) to (o), in addition to the base substitutions (a) to (d) and at least one or more of the base substitutions (e) to (g):

(h) the base T at position 162 is C;
(i) the base T at position 225 is C;
(j) the base T at position 523 is C;
(k) the base T at position 1565 is C;
(l) the base T at position 1763 is C;
(m) the base T at position 2652 is C;
(n) the base T at position 4052 is C; and
(o) the base T at position 4193 is C.

(4) A fragment of DNA encoding the gene 62 of any one of above (1) to (3), which comprising 10 or more base pairs including the base-substituted portions.

(5) A protein encoded by the gene 62 of the Oka vaccine virus of above (1), which comprises at least the following amino acid substitutions (A) to (D) in the amino acid sequence of SEQ ID No. 1:

(A) the amino acid residue Ser at position 628 is Gly;
(B) the amino acid residue Arg at position 958 is Gly;
(C) the amino acid residue Val at position 1197 is Ala; and
(D) the amino acid residue Ile at position 1260 is Val.

6. The protein of the above (5), which comprises at least one or more of the following amino acid substitutions (E) to (H) in addition to the amino acid substitutions (A) to (D):

(E) the amino acid residue Met at position 99 is substituted with Thr;
(F) the amino acid residue Len at position 446 is Pro;
(G) the amino acid residue Val at position 512 is Ala; and
(H) the amino acid residue Leu at position 1275 is Ser.

(7) A peptide as a part of the protein of the above (5) or (6), which comprises 5 or more amino acid residues including the amino acid-substituted portions.

(8) An antibody or a cytotoxic T lymphocyte prepared by using the protein of the above (5) or (6) or the peptide of the above (7) as an antigen.

(9) An attenuated varicella-zoster virus strain, of which genomic gene 62 is the gene 62 of any one of the above (1) to (3).

(10) A method for identifying the Oka vaccine virus or an attenuated varicella-zoster virus strain, comprising selecting a varicella-zoster virus with the same base substitutions in the gene 62 as the gene 62 of any one of the above (1) to (3).

(11) A method for identifying the Oka vaccine virus or an attenuated varicella-zoster virus strain, comprising selecting a varicella-zoster virus carrying genomic DNA to which the DNA fragment of the above (4) hybridizes.

(12) A method for identifying the Oka vaccine virus or an attenuated varicella-zoster virus strain, comprising selecting a varicella-zoster virus producing an antigen recognized by the antibody or the cytotoxic T lymphocyte of the above (8).

(13) A method for identifying the Oka vaccine virus or an attenuated varicella-zoster virus strain, comprising digesting a DNA fragment comprising at least from 2107th to 2229th in the nucleotide sequence of SEQ ID No. 1 by using restriction enzymes *Nae*I and *Bss*HII, and selecting a varicella-zoster virus from which the DNA fragment is digested into 2 or 3 fragments.

(14) A method according to the above (13), wherein the DNA sequence is prepared by PCR using the oligonucleotides of the nucleotide sequence of SEQ ID No. 2 and 3 as primers and varicella-zoster virus gene 62 as a template.

(15) An attenuated varicella-zoster virus strain identified by a method of any one of the above (10) to (14).

(16) A live attenuated vaccone for varicella-zoster prepared by using the attenuated varicella-zoster virus strain of the above (9) or (15) as a seed virus.

Brief Description of Drawings

[0012]

Fig. 1, upper column depicts the schematic view of the structure of varicella virus gene 62; Fig. 1, lower column collectively depicts the sequencing results of the 9 clones derived from the gene 62 of Oka vaccine virus and the sequencing results of the gene 62 of the virulent parental strain.
Fig. 2 is the agarose gel electrophoresis charts of the RFLP results of the PCR products of the gene 62 of each of the following varicella-zoster virus strains: Lane 1 for the Oka vaccine virus; Lane 2 for the highly toxic parental strain; Lane 3 for Kawaguchi strain; Lanes 4 to 8 for varicella-derived strain; Lanes 9 to 13 for zoster-derived strain; the lanes on both the sides are for size markers.
Fig. 3 depicts the results of CAT assay to test transcactivation activity of gene 62 products (IE62) of the Oka vaccine virus and virulent Oka parental strain on individual promoters; Fig. 3A shows that IE62s expressed from the effector plasmids transactivate the gene 4 (IE4) promoter in a dose-dependent fashion, wherein the CV1 cell is cotransfected with pCAT-IE4 at a constant amount (0.25μg) and pVac-G62 (open square) or pPar-G62 (closed circle) at various amounts; and Fig. 3B, C, D, E and F show the activities of IEG2s on the individual promoters of the gene 62 coding for IE62, the gene 28 for DNA polymerase, the gene 29 for major DNA binding protein (abbreviated as MDBP), the gene 14 for glycoprotein C (abbreviated as gC) and the gene 68 for gE, respectively.

Best Mode for Carrying Out the Invention

[0013] Varicella virus gene 62 is one of the gene responsible for the transactivation function (function for activating transcription in the trans-form) and locates in the region of the base numbers 105142 to 109242 in the whole sequence of the varicella virus genome (Journal of General Virology, 67: 1759-1816, 1986). As shown in the upper column of Fig. 1, a transcription starts from the transcription start site along the arrow direction, so the nucleotide strand of the base numbers 109242 to 105142 in the whole genome sequence serves as "+" strand in the gene 62. Thus, the first base "A" in the SEQ ID No. 1 corresponds to the 5' terminal base (base at position 109242 in the whole genome sequence)

of the + strand. In the following description, furthermore, base number is sometimes described as the base number in the whole genome sequence. Because the sequence of the gene 62 in the whole genome sequence is "-" strand, however, the gene 62 is complementary to the nucleotides shown in SEQ ID No. 1.

[0014] The gene 62 of Oka vaccine virus of the invention is a gene with the base substitutions (a) to (d) shown in Table 1 in the nucleotide sequence of SEQ ID No. 1. Otherwise, the gene 62 of Oka vaccine virus is a gene with at least one or more of the base substitutions (e) to (g) in Table 1 in addition to the base substitutions (a) to (d), or the gene additionally with at least one or more of the base substitutions (h) to (o) in Table 1.

[0015] The protein of the attenuated varicella virus of the invention is encoded by the gene 62, wherein the protein is with the amino acid substitutions (A) to (D) shown in Table 1 in the amino acid sequence of SEQ ID No. 1. Otherwise, the protein is with the amino acid substitutions of at least one or more of (E) to (H) shown in Table 1, in addition to the amino acid substitutions (A) to (D).

Table 1

Base substitutions	Amino acid substitutions
(a) 2110th A → G	(A) 661st Ser → Gly
(b) 3100th A → G	(B) 958th Arg → Gly
(c) 3818th T → C	(C) 1197th Val → Ala
(d) 4006th A → G	(D) 1260th Ile → Val
(e) 1251st A → G	
(f) 2226th A → G	
(g) 3657th A → G	
(h) 162th T → C	
(i) 225th T → C	
(j) 523rd T → C	(E) 99th Met → Thr
(k) 1565th T → C	(F) 446th Leu → Pro
(l) 1763rd T → C	(G) 512nd Val → Ala
(m) 2652nd T → C	
(n) 4052nd T → C	(H) 1275th Leu → Ser
(o) 4193rd T → C	

[0016] It is additionally suggested that the Oka vaccine virus carrying the gene 62 with the base substitutions causing such amino acid substitutions is attenuated probably due to the overall reduction of the expression of a protein required for the formation of virus particle because of the defection or attenuation of the transactivator function.

[0017] Such gene 62 of Oka vaccine virus can be isolated for example from the known Oka vaccine virus (BIKEN Lot-65: The Research Foundation of Microbial Diseases of Osaka University or ATCC VR-795) in a conventional manner. Otherwise, such Oka vaccine virus gene 62 can be isolated from an attenuated varicella-zoster virus strain identified by a method as described below.

[0018] The fragment of DNA of the present invention is that encodes the gene 62 set forth above, and comprises 10 or more base pairs including the base substitutions. The DNA fragment can be prepared by cleaving the genomic DNA of Oka vaccine virus or an attenuated varicella-zoster virus strain newly identified by the inventive method with appropriate restriction enzymes. The DNA fragment can be used for example as DNA probe for differentiating the Oka vaccine virus or the attenuated varicella-zoster virus strain from the highly toxic parental strain or other wild-type varicella-zoster virus strains.

[0019] Additionally, the protein of the present invention can be prepared by expressing the gene 62 in microorganisms for example *Escherichia coli* or yeast. More specifically, a DNA fragment carrying the coding region of the gene 62 is inserted in an expression vector with an origin replicable in microorganisms, a promoter, a ribosome-binding site, a DNA -cloning sites and a terminator to prepare an expression vector, to thereby transform a host cell; by subsequently culturing the resulting transformant, the protein encoded by the gene 62 can be produced in microorganisms at a mass scale. Otherwise, the gene 62 can be expressed in the form of a fusion protein with other proteins. By cleaving the

resulting fusion protein with an appropriate protease, only the objective protein can be recovered.

[0020] These proteins can be used as antigens for preparing for example antibody and CTL.

[0021] The peptide of the present invention is of a sequence comprising 5 or more amino acid residues including the amino acid substitutions in the amino acid sequence of the protein. These peptides can be used as antigens for preparing antibodies and CTL.

[0022] The antibody of the present invention can be prepared in the form of polyclonal antibody or monoclonal antibody using the protein per se or a partial peptide thereof as an antigen by a known method. Additionally, the CTL of the prepsent invention can be prepared by using the protein per se or a partial peptide thereof as an antigen by a known method. These antibodies and CTL can be used for the method for identifying a newly isolated attenuated varicella-zoster virus strain.

[0023] The attenuated varicella-zoster virus strain of the invention have the identical base substitutions compared with those of the gene 62 of Oka vaccine virus. Therefore, the attenuated varicella-zoster virus strain of the invention is acceptable as a virus strain for live attenuated varicella vaccine. Such new attenuated varicella-zoster virus strain can be identified by selecting a varicella virus carrying the base substitutions in gene 62. More specifically, such new attenuated varicella-zoster virus strain can be differentiated from the wild-type varicella-zoster virus strain by the method using for example the DNA fragment or the antibody or CTL.

[0024] Otherwise, the new attenuated varicella-zoster virus strain can be identified by the method proposed in this application, namely the method comprising cleaving a DNA sequence comprising at least from 2107th to 2229th in the nucleotide sequence of SEQ ID No. 1 by using restriction enzymes *Nae*I and *Bss*HII, and selecting a varicella virus from which the DNA sequence is cleaved into 2 to 3 fragments. As has been described above, the base A at position 2110 in the gene 62 of Oka vaccine virus is substituted with G. Additionally, the base A at position 2226 is sometimes substituted with G. Consequently, the base substitution at position, an A vs. G, results in the creation of a restriction enzyme *Nae*I site (GCCGGC) at positions 2107 to 2112 in SEQ ID No. 1. Like that, the base substitution at position 2226, an A vs. G, makes *Bss*HII site (GCGCGC) at positions 2224 to 2229 in SEQ ID No. 1. In case that the DNA fragment at least comprising the nucleotides from 2107th to 2229th in SEQ ID No. 1 is cleaved with the two restriction enzymes, hence, the DNA fragment is cleaved into 2 or 3 fragments when the DNA fragment is derived from the Oka vaccine virus or the attenuated varicella virus. In case that the DNA sequence is derived from a wild-type varicella virus or the virulent parental strain of the Oka vaccine virus, while, the DNA sequence is never cleaved by either restriction enzymes, and remains as a single fragment because of no presence of such base substitutions as described above. The DNA fragment cleaved by the restriction enzymes can be prepared by PCR using the oligonucleotides, the nucleotide sequence of SEQ ID No. 2 and No. 3, as primers and the varicella virus gene 62 as an identification subject as template.

[0025] The live attenuated varicella vaccine of the present invention is prepared by using the attenuated varicella-zoster virus strain newly identified by the method described above as the seed virus. Such live vaccine can be prepared in the same manner as the method for preparing conventional live attenuated varicella vaccines using the Oka vaccine virus as the seed virus.

Examples

[0026] The invention will now be described specifically in detail in the following Examples but the invention is never limited to the following examples.

Example 1

[0027] The nucleotide sequence of the gene 62 of Oka vaccine virus and the gene 62 of the highly toxic parental strain were determined. The Oka vaccine virus (live vaccine strain), Biken Lot-65: The research Foundation for Microbial Diseases of Osaka University was used. The virulent parental strain (wild-type Oka vaccine virus) was propagated in MRC-5 cells, and was then used.

[0028] According to the method described in the Example 1 of the previous invention (WO 97/43420), more specifically, genomic DNA were extracted from the Oka vaccine virus and the virulent parental strain, and each DNA was amplified by PCR using primers designed by refering to the nucleotide sequence of the Dumas strain (J. Gen. Virol., 67: 1759-1816, 1986). The entire gene 62 was amplified as three entire overlapping pieces using three sets of primer pair (sense primers G62N1, G62N2 and G62N3 and antisense primers G62R1, G62R2 and G62R3) as shown in Table 2. Each sense primer had M13 forward (-38) sequence (nucleotides at positions 1 to 19 in G62N1-3) at their 5' end, and each antisense primer had M13 reverse sequence (nucleotides at positions 1 to 20 in G62R1-3) linked at their 5' end.

Table 2

Primers	Sequence	Genomic Position
G62N	5'-CCGAGCTCGAATT/GTAGATTCATAAAAACCGTTCCGC-3'	105103-105139
*Xba*I cleavage site		
G62N1	5'-TTTCCCAGTCACGACGTTGTTCATAAAAACCGTTCCGC-3'	105121-105139
G62N2	5'-TTTCCCAGTCACGACGTTGCAGGCACAACCGGTTACTCAG-3'	106455-106475
G62N3	5'-TTTCCCAGTCACGACGTTGTTTGGTCTTACGAATCCTCGG-3'	107844-107864
G62R	5'-ACCTGATCAGAATTCTGCA/GAGCGGTCTCTCCTTAAACGC-3'	109381-109362
*Pst*I cleavage site		
G62R1	5'-GGATAACAATTTCACACAGGTTCTGATCATCTACGATCCG-3'	106600-106581
G62R2	5'-GGATAACAATTTCACACAGGCAAATTCGGATGATTCGGAC-3'	107950-107931
G62R3	5'-GGATAACAATTTCACACAGGAGCGGTCTCTCCTTAAACGC-3'	109381-109362

[0029] Each PCR reaction consisted of 200 mM of deoxynucleotide triphosphate [each], 0.3 μM of each primer, an extremely low amount of template DNA, and 2.5 U of Ex Taq (manufactured by TaKaRa) in 50 μl of Ex Taq buffer. One set of primer pairs (SEQ ID Nos. 3 and 8) included 6 % DMSO in the reaction mixture. Amplification was carried out at 30 cycles of denaturation (94 °C for one minute), annealing (55 °C for 1.5 minutes) and extension (72 °C for 2 minutes), using thermal cycler (Perkin-Elmer, USA).

[0030] All PCR products were purified using the PCR purification kit (QIAGEN GmbH, Germany) to remove primers. Direct sequence of the purified PCR products was performed with a sequencing kit (Amersham, Co., UK) using M13 forward primer or reverse primer labeled with a fluorescent dye IRO-40, analyzed using a DNA sequencer Model 4000L (LI-COR Co., USA).

[0031] By the same method, the DNA sequence of genes 4, 14 and 61 of Oka vaccine virus and the virulent parental strain were determined.

[0032] Consequently, there were no nucleotide change in the genes 4, 14 and 61 between the Oka vaccine virus and the virulent parental strain. As shown in Table 3, 15 bases differences were found in the gene 62 between Oka vaccine virus and the virulent parental strain. Every base substitutions was a T vs C or an A vs G. At eight positions, the nucleotide changes found in the vaccine virus resulted from mixture of two kinds of nucleotide at a single position (R in Table 3). From the respect of 9 bases, additionally, the gene 62 of each of the Oka vaccine virus and the virulent parental strain was different from the gene 62 of the Dumas strain.

[0033] Based on the aforementioned results, it was confirmed that the attenuated Oka vaccine virus could be differentiated from the virulent parental strain in terms of the sequence difference of gene 62.

Table 3

Genomic Position	Vaccine Strain	Parental Strain	Dumas Strain
105169	R (noncoding)	A (noncoding)	A
105310	R (Ser/Leu)	A (Leu)	A
105356	C (Val)	T (Ile)	T
105451	G	G	A
105512	C	C	A
105544	G (Ala)	A (Ala)	A
105705	C (Ala)	T (Ala)	T
106262	C (Gly)	T (Arg)	T
106710	R (Ala)	A (Ala)	A
107136	C (Ala)	T (Ala)	T

Table 3 (continued)

Genomic Position	Vaccine Strain	Parental Strain	Dumas Strain
107165	T	T	C
107252	C (Gly)	T (Ser)	T
107303	C	C	T
107599	R (Ala/Val)	A (Val)	A
107607	A	A	C
107715	C	C	T
107797	R (Pro/Leu)	A (Leu)	A
108111	C (Pro)	T (Pro)	T
108747	G	G	A
108838	R (Thr/Met)	A (Met)	A
108951	A	A	G
109044	G	G	C
109137	R (silent)	A (silent)	A
109200	R (silent)	A (silent)	A

Example 2

[0034] The entire gene 62 was cloned from Oka vaccine virus and the virulent parental strain by PCR.

[0035] PCR conditions were same as in Example 1 except for the use of the oligonucleotides G62N and G62R in Table 1 as primer pairs, the addition of 6 % DMSO in the reaction mixture, and an extension time of 6 minutes for every cycle. The full length of each gene 62 was amplified by PCR.

[0036] Because the primers individually had restriction enzymes *Xba*I and *Pst*I sites, the purified PCR product was digested with these restriction enzymes. And then the digested product was inserted into pUC19, which had been digested in advance with the corresponding enzymes. After the transformation of *E. coli* JM109 with hybrid plasmids, 9 plasmids were extracted from the Oka vaccine and the virulent parental tansformants for DNA sequencing.

[0037] The results are shown in Fig. 1. As shown in Fig. 1, plural bases among the bases at 15 positions in each of the 9 clones of the Oka vaccine virus were different from those of the virulent parental strain. Particularly, the 7 bases at base positions 1251, 2110, 2226, 3100, 3667, 3818 and 4006 in all of the clones were different from the bases at the same positions in the parental strain. No nucleotide changes were observed in any of the 9 clones from the virulent parental strains for these 15 sites.

Example 3

[0038] Partial sequences of the gene 62s of various varicella-zoster virus strains were analyzed by RFLP.

[0039] The Oka vaccine virus and the virulent parental strain were used as way in Example 1. Additionally, the Kawaguchi strain, one of wild-type varicella-zoster virus strains and 10 samples of wild-type virus strains isolated from vesicular fluids from non-vaccinated 5 patients with varicella and 5 patients with zoster were used. Herein, the Kawaguchi strain and the wild-type varicella-zoster virus strains isolated from these patients were propagated in HEL cell or MRC-5 cell over 2 to 3 times, arid were then used.

[0040] The gene 62 of each varicella virus was amplified by PCR using the oligonucleotides of SEQ ID Nos. 2 and 3 as primers. The oligonucleotide of SEQ ID No. 2 was the reverse primer and corresponding to the bases at positions 1846 to 1863 in the varicella virus gene 62 (SEQ ID No. 1), while the oligonucleotide of SEQ ID No. 3 was the forward primer corresponding to the bases at positions 2609 to 2620 thereof. PCR conditions were the same as in Example 1, except that the extension time was one minute for every cycle. Three microliters of each PCR product were digested sequentially with 4 U *Nae*I (TaKaRa) at 37 °C for 1.5 hours and 4U *Bss*HII (TaKaRa) at 50 °C for 1.5 hours. The digested DNA fragments were analyzed by electrophoresis on 4 % agarose gel (NuSieve 3:1, FMC BioProducts, Co.) with ethidium bromide staining.

[0041] The results are shown in Fig. 2. As apparently shown in Fig. 2, the PCR product (Lane 1) of the gene 62 from Oka vaccine virus was cleaved into three fragments (402 bp, 264 bp and 116 bp) by *Nae*I and *Bss*HII, whereas the PCR

products (Lanes 2-13) of the virulent parental strain and the wild-type virus strains isolated from the patients were never digested by either restriction enzymes and remained as a single fragment.

[0042] The aforementioned results establish the confirmation that by cleaving a DNA sequence at least comprising the base at position 2107 to 2229 in the varicella virus gene 62 with restriction nucleases *Nae*I and *Bss*HII, a varicella virus with a DNA sequence thereby cleaved into 2 to 3 fragments could be identified as the Oka vaccine virus or a virus strain acceptable as attenuated varicella virus.

Example 4

[0043] So as to analyze the correlation between the eight amino acid mutations ad the transactivation activities f the gene 62 products, IE62, chloramphenicol transferase (CAT) assays were performed in cells cotransfected with a reporter plasmid having a promoter sequence of each VZV gene upstream of CAT gene and an effector plasmid carrying the gene 62 of Oka vaccine virus or the highly toxic parental strain.

1. Materials and Methods

1.1 Plasmids

[0044] Reporter plasmids pIE4-CAT, pgC-CAT, pPOL-CAT, pMDBP-CAT, pIE62-CAT and pgE-CAT contained about 750 bases upstream of the initiation codon the promoter region of genes 4, 14, 28, 29, 62 and 68, respectively. Each promoter region was amplified from the Oka vaccine strain by PCR using specific primers which had *Nhe*I- or *Bgl*II site at the 5' end. Conditions of PCR were the same as in Example 1, except for the extension time of one minute. The amplified products were digested with *Nhe*I and *Bgl*II, and were then inserted into upstream of CAT gene of pCAT3-Basic plasmid (Promega, Co., USA).

[0045] Effector plasmid pPar-G62 was selected among 9 clones from gene 62 clone of the virulent parental strain. Clone 9 of Fig. 1 (the clone containing 13 base substitutions and 8 amino acid substitutions) was selected from among the 9 clones from the Oka vaccine strain and uses as the effector plasmid, pVac-G62.

1.2 Transfection

[0046] CV1 cells were cultured in a 35-mm plastic dish at 10^5 cells/dish and were transfected by lipofection using Superfect (QIAGEN GmbH). Each reaction mixture for transfection consisted of 0.25 μg of reporter plasmid and various amounts (0-1 μg) of either effector plasmid . The total DNA amount in each transfection was kept constant at 2.5 μg by adding the vector pUC19. All experiments were repeated at least three times, with independent DNA transfection.

1.3 CAT assay

[0047] Forty four hours after transfection, the total protein and CAT levels in the cell were assayed. The cell was washed three times with phosphate-buffered saline, and then lysed in lysis buffer (Boehringer Mannheim Corporation, Germany). The CAT concentration of the individual lysate was determined by a CAT ELISA kit (Boehringer Mannheimn Corporation, Germany) and was standardized to each protein concentration, that was determined by the Bio-Rad protein reagent (Bio-Rad, USA).

2. Results

[0048] All of the VZV gene promoters tested displayed very low-level basal activity; little or no CAT expression was observed when no effector plasmid was transfected.

[0049] The gene 4 promoter derived from Oka strain was transactivated by both gene 62 products (IE62s) of the virulent parental strain and the Oka strain (Fig. 3A). The transactivation activity of IE62 of the virulent parental strain was stronger than that of the IE62 by the Oka vaccine strain. When the lowest dose (0.25 μg) of the effector plasmids was transfected, the activity of the virulent parental strain IE62 was 7.8-fold higher than that by the Oka strain IE62. As the effector concentration was increased, the difference in the activity of the two IE62 proteins diminished.

[0050] Little CAT could be detected in the cells transfected with the reporter plasmid pCAT-IE62 (Fig. 3B).

[0051] Furthermore, pCAT-Pol and pCAT-MDBP carrying the VZV genes 28 and 29 promoters, respectively (Fig. 3C and 3D), and pCAT-gE carrying the promoter sequence of the gene 68 (Fig. 3F) were activated by both IE62s from the virulent parental strain and the Oka vaccine strain. Each dose-dependent curves was similar to the pCAT-IE4 response, and the activity of the virulent parental strain IE62 was higher than that of the Oka vaccine strain IE62 at all dose. At the lowest dose of effector plasmids, in particular, the activity of the virulent parental strain IE62 was 7.6-fold, 5.6-fold and

1.8-fold higher than that of the Oka vaccine strain IE62. Little CAT activity was detected in the cell transfected with pCAT-gC carrying the promoter region of the gene 14 (Fig. 3E).

[0052] The aforementioned results establish the confirmation that the expression product (IE62) of the VZV gene 62 activated the promoters of the pre-early gene 4 (ORF4), early gene 28 (DNA polymerase: Pol) and gene 29 (major DNA binding protein: MDBP) and delayed gene 68 (glycoprotein: gE). It is additionally of more importance that the transcription activity of the Oka vaccine strain IE62 was constantly lower than that of the highly toxic parental strain IE62. In other words, the mutations in the gene 62 of the Oka vaccine strain have some influence on virus replication, which induces the attenuation of VZV.

Industrial Applicability

[0053] In accordance with the present invention, the Oka vaccine virus or a varicella-zoster virus strain acceptable as attenuated varicella vaccine can be accurately discriminated in a simple manner from other wild-type varicella viruses and the highly toxic parental strain of the Oka vaccine virus. In accordance with the present invention, furthermore, attenuated varicella virus antigens for live vaccine can be prepared at a mass scale. Owing to the inventions, vaccine preparation and the safety and effectiveness of vaccination can be achieved in a more reliable manner.

Sequence Listing

<110> The Research Foundation for Microbial Diseases of Osaka University

<120> Gene 62 of Oka vaccine virus and method for identifying virus strain for live attenuated vaccine virus using the gene 62.

<140> PCT/JP99/05476

<141> 1999-10-05

<150> JP11-48964

<151> 1999-02-25

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<212> DNA

<213> Varicella-Zoster virus

<220>

<221> CDS

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ACACGTTTTA AGTACTGTTG GAACTCCCTC ACCAACCGCA ATCGCAATCC TTTGAAGGCT  180
GCGAGAGCGT TTGGAAAACT CGGGTACGTC TAAATTCACC CCAGTGCG ATG GAT       234
                                                    Met Asp
                                                     1
ACG CCG CCG ATG CAG CGC TCT ACA CCC CAA CGC GCG GGG TCG CCT GAT     282
Thr Pro Pro Met Gln Arg Ser Thr Pro Gln Arg Ala Gly Ser Pro Asp
  5                  10                  15
ACT TTG GAG TTA ATG GAC CTG TTG GAC GCG GCC GCC GCG GCC GCC GAA     330
Thr Leu Glu Leu Met Asp Leu Leu Asp Ala Ala Ala Ala Ala Ala Glu
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                20                  25                  30
CAC AGG GCC CGG GTG GTC ACC TCG AGT CAG CCT GAC GAT CTA CTA TTT   378
His Arg Ala Arg Val Val Thr Ser Ser Gln Pro Asp Asp Leu Leu Phe
 35                  40                  45                  50
GGA GAG AAC GGG GTC ATG GTG GGA CGG GAA CAT GAG ATC GTT TCA ATT   426
Gly Glu Asn Gly Val Met Val Gly Arg Glu His Glu Ile Val Ser Ile
                    55                  60                  65
CCC TCC GTA TCG GGA CTT CAA CCA GAA CCC AGA ACG GAA GAT GTT GGC   474
Pro Ser Val Ser Gly Leu Gln Pro Glu Pro Arg Thr Glu Asp Val Gly
            70                  75                  80
GAA GAG CTA ACA CAA GAC GAC TAC GTA TGC GAG GAC GGT CAG GAT CTA   521
Glu Glu Leu Thr Gln Asp Asp Tyr Val Cys Glu Asp Gly Gln Asp Leu
        85                  90                  95
ATG GGC TCG CCT GTA ATC CCG CTG GCC GAG GTC TTC CAC ACC CGA TTC   570
Met Gly Ser Pro Val Ile Pro Leu Ala Glu Val Phe His Thr Arg Phe
    100                 105                 110
TCG GAG GCC GGC GCG CGA GAA CCA ACA GGA GCC GAT CGC TCC CTC GAG   618
Ser Glu Ala Gly Ala Arg Glu Pro Thr Gly Ala Asp Arg Ser Leu Glu
115                 120                 125                 130
ACA GTC TCT CTC GGA ACG AAG CTT GCT AGG TCT CCA AAA CCA CCG ATG   666
Thr Val Ser Leu Gly Thr Lys Leu Ala Arg Ser Pro Lys Pro Pro Met
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AAC GAT GGG GAA ACG GGC AGA GGT ACG ACC CCT CCG TTC CCG CAG GCC   714
Asn Asp Gly Glu Thr Gly Arg Gly Thr Thr Pro Pro Phe Pro Gln Ala
            150                 155                 160
TTC TCC CCT GTA TCC CCC GCG TCT CCT GTT GGA GAC GCC GCC GGG AAC   762
Phe Ser Pro Val Ser Pro Ala Ser Pro Val Gly Asp Ala Ala Gly Asn
        165                 170                 175
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Asn Ser Pro Gly Leu Pro Ser Val Val His Arg Asp Arg Gln Thr Gln
195                 200                 205                 210

TCC ATC TCG GGT AAA AAG CCG GGC GAT GAG CAA GCG GGT CAT GCG CAT    906
Ser Ile Ser Gly Lys Lys Pro Gly Asp Glu Gln Ala Gly His Ala His
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Ala Ser Gly Asp Gly Val Val Leu Gln Lys Thr Gln Arg Pro Ala Gln
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        245                 250                 255

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Ala Arg Lys Pro Gly Gly Pro Val Pro Gly Pro Val Glu Gln Leu Tyr
    260                 265                 270

CAC GTC CTT TCG GAC AGC GTT CCC GCT AAG GGG GCA AAG GCG GAC CTG   1098
His Val Leu Ser Asp Ser Val Pro Ala Lys Gly Ala Lys Ala Asp Leu
275                 280                 285                 290

CCG TTT GAG ACC GAT GAT ACC CGC CCA AGG AAA CAT GAT GCC CGG GGT   1146
Pro Phe Glu Thr Asp Asp Thr Arg Pro Arg Lys His Asp Ala Arg Gly
                295                 300                 305

ATA ACA CCT CGC GTC CCT GGA CGT TCG TCG GGG GGC AAA CCT AGA GCG   1194
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TTT TTG GCC CTG CCG GGA AGA TCC CAC GCA CCA GAC CGG ATT GAG GAT   1242
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TCA TCC TCT TCC TCG TCG TGG GGA TCG TCA TCG GAG GAT GAA GAC GAT   1338
Ser Ser Ser Ser Ser Ser Trp Gly Ser Ser Ser Glu Asp Glu Asp Asp
355                 360                 365                 370

GAA CCC CGG CGC GTT TCG GTG GGA AGT GAA ACT ACA GGC AGC AGG TCC   1386
Glu Pro Arg Arg Val Ser Val Gly Ser Glu Thr Thr Gly Ser Arg Ser
                375                 380                 385

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            390                 395                 400

AAT GAT GGT GGG TCG ACG AAA CAA AAT ATC CAA CCG GGA TAT CGA TCC   1482
Asn Asp Gly Gly Ser Thr Lys Gln Asn Ile Gln Pro Gly Tyr Arg Ser
        405                 410                 415

ATC AGC GGT CCC GAT CCG AGG ATT CGT AAG ACC AAA CGT CTT GCG GGG   1530
Ile Ser Gly Pro Asp Pro Arg Ile Arg Lys Thr Lys Arg Leu Ala Gly
    420                 425                 430

GAA CCG GGG CGC CAG AGA CAG AAA TCA TTT TCC CTG CCG CGA TCC AGA   1578
Glu Pro Gly Arg Gln Arg Gln Lys Ser Phe Ser Leu Pro Arg Ser Arg
435                 440                 445                 450

ACC CCG ATA ATT CCC CCG GTG TCG GGG CCG CTC ATG ATG CCC GAC GGA   1626
Thr Pro Ile Ile Pro Pro Val Ser Gly Pro Leu Met Met Pro Asp Gly
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TAC GTG CCG GAG TTG GGA GAT CCG GCT AGA CAG TAC CGC GCG CTG ATT  1818
Tyr Val Pro Glu Leu Gly Asp Pro Ala Arg Gln Tyr Arg Ala Leu Ile
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Asn Leu Ile Tyr Cys Pro Asp Arg Asp Pro Ile Ala Trp Leu Gln Asn
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CCC AAG CTG ACC GGT GTC AAC TCG GCC CTG AAC CAG TTC TAC CAA AAG  1914
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Ser Pro Val Pro His Val Gly Glu Ala Met Ala Thr Gly Glu Ala Leu
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Asp Pro Gln Pro Ser Ala Ala Ala Arg Ser Leu Ser Val Cys Pro Pro

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725 730 735

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Ile Thr Met Pro Leu Asp Gly Pro Ala Pro Asn Gly Gly Phe Arg Arg

740 745 750

ATT CCC CGG GGG GCC CTG CAT ACC CCG GTC CCG TCG GAC CAG GCT CGC 2538

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Val Leu Ala Ala Leu Ser Asn Arg Leu Cys Leu Pro Ser Thr His Ala
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            950                 955                 960
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995                 1000                1005                1010
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His Arg Ala Ala Asn Arg Trp Gly Leu Gly Ala Ala Leu Arg Pro Val

1095 1100 1105

TTC CTT CCC GAG GGA CGG AGA CCG GGG GCC GCC GGG CCG GAG GCC GGC 3594

Phe Leu Pro Glu Gly Arg Arg Pro Gly Ala Ala Gly Pro Glu Ala Gly

1110 1115 1120

GAC GTA CCC ACC TGG GCG AGG GTG TTT TGC CGC CAC GCC CTG CTG GAA 3642

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1125 1130 1135

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1175 1180 1185

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Val Leu Glu Gly Ser Asp Gly Thr Arg Phe Val Phe Gly His His Gly

1190 1195 1200

GGC TCG GAA CGG CCG GCA GAA ACC CAG GCG GGG CGA CAG CGG CGC ACC 3882

Gly Ser Glu Arg Pro Ala Glu Thr Gln Ala Gly Arg Gln Arg Arg Thr

1205 1210 1215

GCA GAC GAC AGA GAA CAC GCT TTG GAG CCG GAC GAT TGG GAG GTG GGG 3930

Ala Asp Asp Arg Glu His Ala Leu Glu Pro Asp Asp Trp Glu Val Gly

1220 1225 1230

TGT GAA GAC GCG TGG GAC AGC GAG GAG GGG GGC GGG GAC GAC GGG GAC 3978

Cys Glu Asp Ala Trp Asp Ser Glu Glu Gly Gly Gly Asp Asp Gly Asp

1235 1240 1245 1250

GCA CCG GGG TCA TCC TTT GGG GTG AGC ATC GTG TCG GTG GCC CCG GGT 4026

Ala Pro Gly Ser Ser Phe Gly Val Ser Ile Val Ser Val Ala Pro Gly

1255 1260 1265

GTG CTG CGA GAC CGC CGG GTG GGC TTG CGC CCG GCG GTC AAG GTG GAG 4074

Val Leu Arg Asp Arg Arg Val Gly Leu Arg Pro Ala Val Lys Val Glu

1270 1275 1280

CTG TTG TCC TCG TCC TCG TCC TCC GAG GAC GAG GAC GAT GTG TGG GGA 4122

Leu Leu Ser Ser Ser Ser Ser Ser Glu Asp Glu Asp Asp Val Trp Gly

1285 1290 1295

GGG CGC GGG GGG AGG AGC CCC CCG CAG AGT CGG GGG TGACGGAGTC 4168

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<210> 3

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<400> 3

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Claims

1. Gene 62 of Oka vaccine virus, which comprises at least the following base substitutions (a) to (d) in the nucleotide sequence of SEQ ID No. 1:

   (a) the base A at position 2110 is G;
   (b) the base A at position 3100 is G;
   (c) the base T at position 3818 is C; and
   (d) the base A at position 4006 is G.

2. The gene 62 of the Oka vaccine virus of claim 1, which comprises at least one or more of the following base substitutions (e) to (g) in addition to the base substitutions (a) to (d):

   (e) the base A at position 1251 is G;
   (f) the base A at position 2226 is G; and
   (g) the base A at position 3657 is G.

3. The gene 62 of the Oka vaccine virus of claim 2, which comprises at least one or more of the following base substitutions (h) to (o), in addition to the base substitutions (a) to (d) and at least one or more of the base substitutions (e) to (g):

   (h) the base T at position 162 is C;
   (i) the base T at position 225 is C;
   (j) the base T at position 523 is C;
   (k) the base T at position 1565 is C;
   (l) the base T at position 1763 is C;
   (m) the base T at position 2652 is C;
   (n) the base T at position 4052 is C; and
   (o) the base T at position 4193 is C.

4. A fragment of DNA encoding the gene 62 of any one of claims 1 to 3, which comprising 10 or more base pairs including the base-substituted portions.

5. A protein encoded by the gene 62 of the Oka vaccine virus of claim 1, which comprises at least the following amino acid substitutions (A) to (D) in the amino acid sequence of SEQ ID No. 1:

   (A) the amino acid residue Ser at position 628 is Gly;
   (B) the amino acid residue Arg at position 958 is Gly;
   (C) the amino acid residue Val at position 1197 is Ala; and
   (D) the amino acid residue Ile at position 1260 is Val.

6. The protein of claim 5, which comprises at least one or more of the following amino acid substitutions (E) to (H) in addition to the amino acid substitutions (A) to (D):

   (E) the amino acid residue Met at position 99 is Thr;

(F) the amino acid residue Leu at position 446 is Pro;
(G) the amino acid residue Val at position 512 is Ala; and
(H) the amino acid residue Leu at position 1275 is Ser.

7. A peptide as a part of the protein of claim 5 or 6, which comprises 5 or more amino acid residues including the amino acid-substituted portions.

8. An antibody or a cytotoxic T lymphocyte prepared by using the protein of claim 5 or 6 or the peptide of claim 7 as an antigen.

9. An attenuated varicella-zoster virus strain, of which genomic gene 62 is the gene 62 of any one of claims 1 to 3.

10. A method for identifying the Oka vaccine virus or an attenuated varicella-zoster virus strain, comprising selecting a varicella-zoster virus with the same base substitutions in the gene 62 as the gene 62 of any one of claims 1 to 3.

11. A method for identifying the Oka vaccine virus or an attenuated varicella-zoster virus strain, comprising selecting a varicella-zoster virus carrying genomic DNA to which the DNA. fragment of claim 4 hybridizes.

12. A method for identifying the Oka vaccine virus or an attenuated varicella-zoster virus strain, comprising selecting a varicella-zoster virus producing an antigen recognized by the antibody or the cytotoxic T lymphocyte of claim 8.

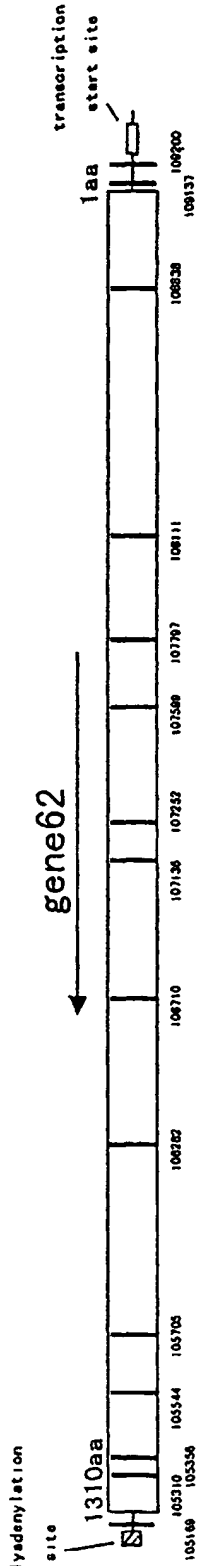
13. A method for identifying the Oka vaccine virus or an attenuated varicella-zoster virus strain, comprising digesting a DNA fragment comprising at least from 2107th to 2229th in the nucleotide sequence of SEQ ID No. 1 by using restriction enzymes *Nae*I and *Bss*HII, and selecting a varicella-zoster virus from which the DNA fragment is digested into 2 or 3 fragments.

14. A method according to claim 13, wherein the DNA sequence is prepared by PCR using the oligonucleotides of the nucleotide sequence of SEQ ID No. 2 and 3 as primers and varicella-zoster virus gene 62 as a template.

15. An attenuated varicella-zoster virus strain identified by a method of any one of claims 10 to 14.

16. A live attenuated vaccone for varicella-zoster prepared by using the attenuated varicella-zoster virus strain of claim 9 or 15 as a seed virus.

Fig. 1



position		105169	105310	105358	105544	105705	106282	106710	107138	107252	107599	107797	108111	108838	109137	109200
Oka parental	all clones	A	A	T	A	T	T	A	T	T	A	A	T	A	A	A
Oka vaccine	clone 1	A	A	C	G	C	C	G	C	C	G	A	C	A	A	G
	clone 2	A	A	C	G	C	C	A	C	C	G	A	C	A	A	A
	clone 3	G	G	C	G	C	C	A	C	C	A	G	C	A	A	G
	clone 4	A	G	C	G	C	C	G	C	C	G	A	C	A	A	G
	clone 5	G	A	C	G	C	C	A	C	C	G	A	C	A	A	A
	clone 6	A	A	C	G	C	C	T	C	C	A	A	C	A	A	G
	clone 7	G	A	C	G	C	C	A	C	C	G	A	C	A	A	A
	clone 8	G	A	C	G	C	C	A	C	C	G	A	C	G	G	A
	clone 9	G	G	C	G	C	C	A	C	C	G	G	C	G	G	A
*amino acid		-	Leu	Ile	Ala	Ala	Arg	Ala	Ala	Ser	Val	Leu	Pro	Met	-	-
		-	Ser	Val	Ala	Ala	Gly	Ala	Ala	Gly	Ala	Pro	Pro	Thr	-	-

Fig. 2

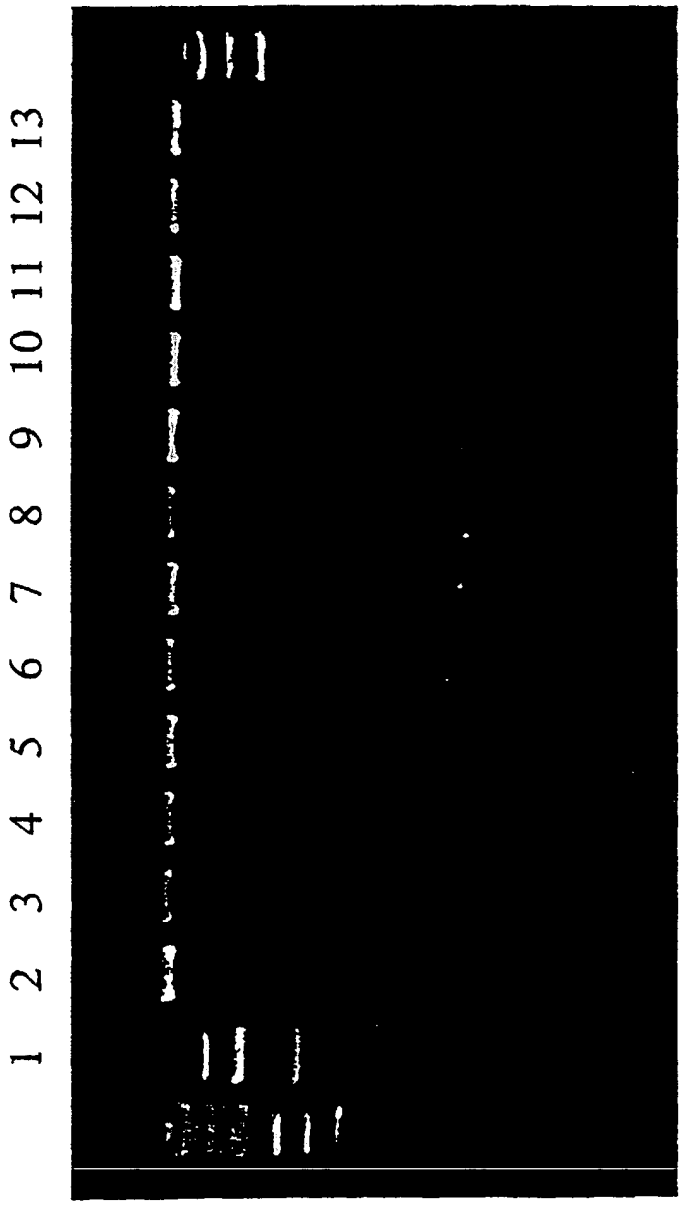
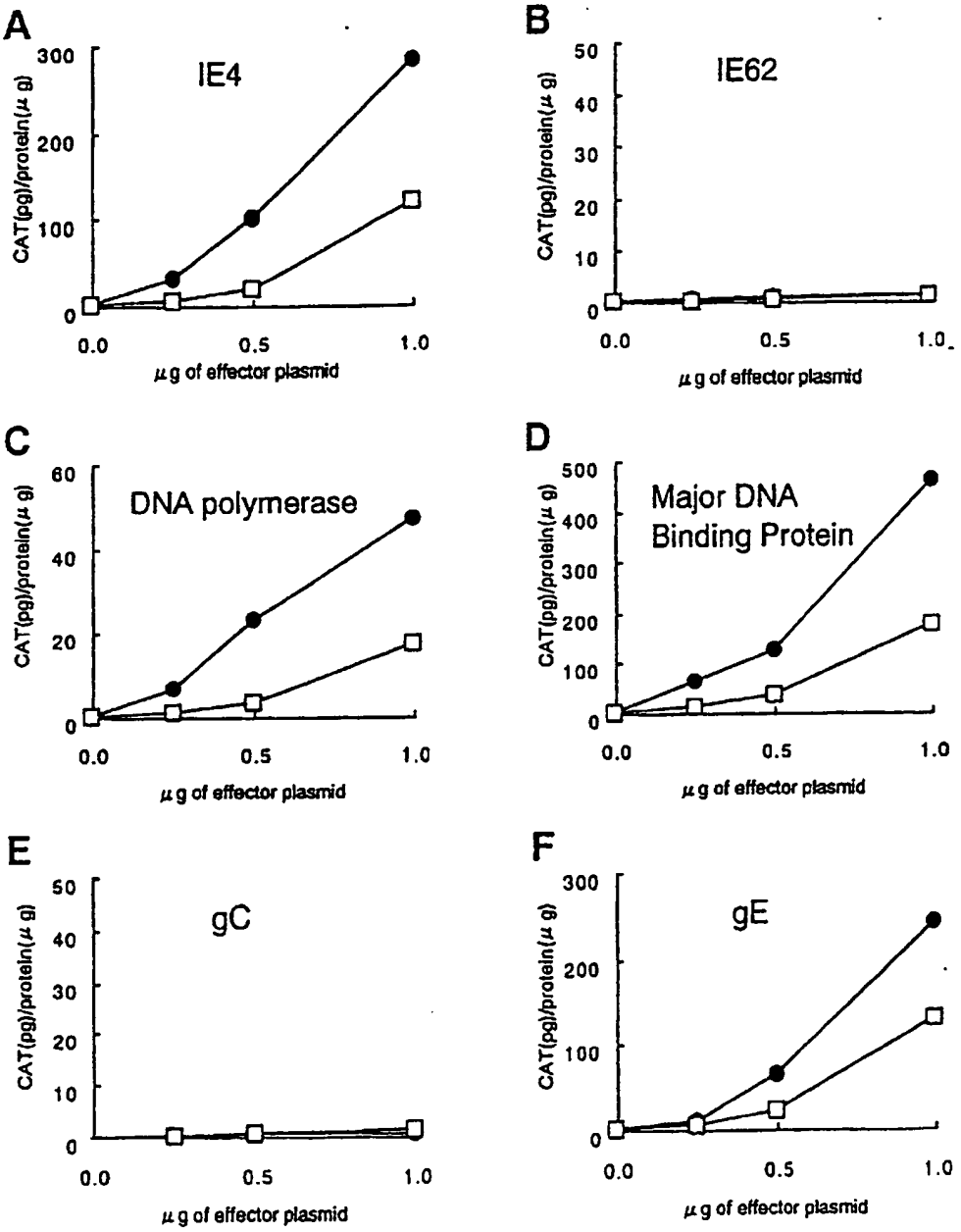


Fig. 3


A
IE4
CAT(pg)/protein(μg)
300
200
100
0
0.0
0.5
1.0
μg of effector plasmid
B
IE62
CAT(pg)/protein(μg)
50
40
30
20
10
0
0.0
0.5
1.0
μg of effector plasmid
C
DNA polymerase
CAT(pg)/protein(μg)
60
40
20
0
0.0
0.5
1.0
μg of effector plasmid
D
Major DNA
Binding Protein
CAT(pg)/protein(μg)
500
400
300
200
100
0
0.0
0.5
1.0
μg of effector plasmid
E
gC
CAT(pg)/protein(μg)
50
40
30
20
10
0
0.0
0.5
1.0
μg of effector plasmid
F
gE
CAT(pg)/protein(μg)
300
200
100
0
0.0
0.5
1.0
μg of effector plasmid

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP99/05476

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] C12N15/38, C12N5/06, C12N7/08, C12P21/08, C12Q1/68, C12Q1/70, C07K14/04, C07K16/08, A61K39/25

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[7] C12N15/38, C12N5/06, C12N7/08, C12P21/08, C12Q1/68, C12Q1/70, C07K14/04, C07K16/08, A61K39/25

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPI (DIALOG), BIOSIS (DIALOG)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	EP, 510996, A1 (RES FOUND MICROBIAL DIS UNIV OSAKA), 28 October, 1992 (28.10.92) & JP, 06-189752, A & AU, 659449, B & US, 5849476, A & CA, 2066998, A & TW, 210354, A	1-4,9,15,16 5-8
X Y	US, 3985615, A (RES FOUND MICROBIAL DIS UNIV OSAKA), 12 October, 1976 (12.10.76) & JP, 53-041202, B & BE, 826568, A & FR, 2263786, A & GB, 1457382, A	1-4,9,15,16 5-8
Y	FORGHANI,B. et al."Monoclonal antibody to immediate early protein encoded by varicella-zoster virus gene 62", Virus Res.(1990), vol.16, No.2, p195-210	5-8
A	WO, 97/43420, A1 (RES FOUND MICROBIAL DIS UNIV OSAKA), 20 November, 1997 (20.11.97) & EP, 839911, A1 & CN, 1193351, A	10-14
A	DAVISON,A.J. et al."The complete DNA sequence of varicella-zoster virus", J.gen.Virol.(1986), vol.67,No.9, p1759-1816	10-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 January, 2000 (24.01.00)	01. 02.00
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